Chapter 4

Table 4.1. Patterns of gene expression regulation that were stimulated by R848 and/or TNFα. Up and down indicates upregulation and downregulation respectively, while No designates no differential regulation of gene expression. UT=no treatment, R= R848, T= TNFα and RT=R848+TNFα. 2-fold change cut off was applied to determine differential expression compared to unstimulated neutrophils. The following tables mention the members of each group of genes related to a particular pattern of transcription regulation indicated in this table. They are presented in the same order of their appearance in this table.

No. of genes	R/UT	T/UT	RT/UT	Comments	Pattern of regulation in RT
38	Up/No	Up/No	Up	RT>R+T	Synergistic upregulation
14	Up	Up	Up	RT=R+T	Additive upregulation
235	Up	Up	Up	RT=R, RT>T	Upregulatory effect dominated by R
33	Up	Up	Up	RT=T, RT>R	Upregulatory effect dominated by T
22	Up	Up	Up	RT=R, RT<T	R prevented further induction by T
4	Up	Up	Up	RT<T, RT>R	R partially prevented further induction by T
19	Up	Up	Up	RT=T, RT<R	T prevented further induction by R
14	Up	No	No		T stopped R-mediated upregulation
5	Up	No	Up	RT<R	T partially stopped R-mediated upregulation
3	Up	Up	No		R and T had different combined effect to their individual effects
43	No	Up	No		R completely inhibited upregulatory effect of T
9	No	Up	Up	RT<T	R partially inhibited upregulatory effect of T
5	Down	Up	Down	RT<T, RT=R	Downregulatory effect due to R alone
38	Up	Down	Up	RT=R	R masked or prevented T effect and increased gene expression
42	No	Down	No	RT=R	R prevented T effect
12	Down	Down	Down	RT=R, RT>T	R stopped further down regulation by T
37	Down	Down	Down	RT<R, RT<T	Additive inhibition
393	Down	Down	Down	RT=R, RT<T	R masked or dominated T effect
25	Down	Down	Down	RT>R, RT≤T	T partially stopped downregulatory effect of R
8	Down	Down	Down	RT=T, RT<R	T masked or dominated R effect
12	Down	No	Down	RT>R, RT<T	T partially stopped downregulatory effect of R
16	Down	No	Down	RT=T	T completely stopped downregulatory effect of R
212	No	No	Down		Only dual action of R and T reduced expression level in U

Table 4.2. Genes synergistically upregulated by R848+TNFα. Molecule types, FPKMs associated with no treatment (UT), R848, TNFα and R848+TNFα treatments, and calculated synergy ratios were indicated.

Gene	Molecule type	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$	Ratio
IL6	Cytokine	0.16	112.85	0.49	194.77	1.72
IL12B		0.36	15.14	1.38	29.24	1.73
IL23A		0.12	17.60	5.60	56.34	2.42
IL27		0.25	1.68	0.64	4.52	1.76
IL36G		0.03	6.00	0.28	22.47	3.56
TNFSF9		0.01	0.51	0.15	5.00	7.34
CXCL10		0.04	13.41	1.20	18.69	1.28
CSF3		0.05	40.06	1.10	86.72	2.10
SOCS3	Enzyme	15.55	65.11	13.68	142.17	1.51
CARD17		0.68	14.50	8.15	30.53	1.31
CD274		0.71	107.52	18.88	164.87	1.30
DHX58		1.83	71.15	2.58	101.52	1.34
PTGS2		15.77	164.76	60.51	321.33	1.33
LSS		0.20	15.13	12.62	39.44	1.41
B3GNT7		0.17	1.61	0.58	4.32	1.84
CASP5		1.12	18.82	5.77	39.44	1.53
MAP3K8		20.79	454.74	143.66	967.87	1.56
PTX3	Receptor	0.09	4.42	2.26	12.87	1.90
NR4A1		0.31	5.67	6.48	15.97	1.28
P2RX4		1.44	11.74	5.58	23.66	1.26
SEMA6A		0.05	4.72	0.75	8.11	1.47
SLAMF7		2.09	273.49	72.49	535.78	1.54
CD200	Membrane-associated protein	0.02	1.18	0.45	3.07	1.86
FAM57A		0.03	1.15	1.45	5.88	2.24
COL17A1		0.05	1.65	0.90	5.38	2.07
FLVCR2		0.12	0.92	0.52	3.26	2.08
IFT57	Transcription factor	0.77	7.37	3.00	15.51	1.39
BATF		1.95	20.23	5.21	40.19	1.47
STAT5A		23.80	63.10	94.90	267.08	1.47
FOXP1		3.14	3.36	1.44	13.82	1.74
CFB	Complement factor	0.01	2.98	1.48	9.07	2.03
TRIP10	Signal transducer	0.35	17.90	19.35	50.13	1.33
TRAF4		0.26	6.56	1.21	16.41	2.04
PI3	Inhibitor	28.47	7373.06	2969.67	15368.90	1.48
SERPINB2		0.24	11.71	2.76	20.36	1.38
SPINK1		1.89	16.40	9.36	38.86	1.41
EIF3CL	Translation factor	0.00	0.00	0.00	3.29	907.10
SMG9	mRNA decay factor	2.43	25.18	11.91	52.73	1.33

Table 4.3. List of 14 TNFα/R848-upregulated genes that were additively induced by the effect of R848 and TNFα. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ABTB2	0.62	2.12	1.91	4.62
BCL2L1	6.34	18.77	16.50	43.94
DNAJB5	0.99	4.80	4.23	10.62
LOC100499177	7.42	20.07	12.20	40.19
MGLL	1.78	18.14	20.01	41.80
PHF19	1.21	7.44	6.37	16.55
RASGRP1	0.30	2.77	2.69	6.55
RHBDF2	2.17	45.07	35.15	96.32
SLC24A1	0.14	5.41	4.49	11.57
SMG7	17.96	27.86	36.08	72.39
TMEM106A	1.12	4.65	3.07	10.17
TMEM38B	0.34	1.55	1.02	3.25
TNFAIP8	18.37	41.91	64.93	134.67
TSEN15	0.21	1.10	0.94	2.60

Table 4.4. A selection of TNFα-stimulated genes (235 in total) that were further stimulated by treatment with R848 and TNFα. The additional induction of gene expression was comparable to R848 effect alone indicating that R848 dominated the effect of TNFα. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ADA	0.98	28.57	11.93	31.06
ARL8B	23.15	107.96	55.24	112.11
C3	0.99	109.81	54.47	111.55
CCL4L2	55.20	7583.38	2512.90	7752.05
CCRL2	28.21	949.51	388.64	1010.01
FXYD6	0.75	23.63	6.71	24.43
G0S2	87.44	3277.50	1687.38	3527.76
GK	42.19	367.17	176.23	378.19
HCAR3	12.92	235.67	89.87	258.33
MAPK6	2.88	73.06	32.10	79.73
MOB3C	4.28	42.68	17.24	46.10
MORF4L2	9.12	42.92	19.64	43.42
PLEK	163.16	1913.05	766.41	2011.78
SLC5A5	2.31	13.55	5.98	14.24
SLC7A11	2.42	12.14	6.20	12.75
STAT4	1.03	29.38	10.69	29.52
TMEM123	54.57	364.77	112.69	367.27
TNF	3.63	431.70	136.16	445.41
WSB1	68.66	364.66	183.30	390.88

Table 4.5. A list of some TNFα/R848-stimulated genes (33 in total) that were further stimulated by the treatment with R848 and TNFα. However, the additional induction of gene expression was comparable to TNFα effect alone indicating that TNFα dominated the effect of TNFα. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	$R848 + TNF\alpha_{FPKM}$
C8orf38	0.18	1.42	4.23	4.22
CD40	0.01	2.85	10.08	10.60
CLEC4D	2.96	11.55	24.25	23.85
DHRS13	6.32	18.69	53.82	47.41
DNAJC3	16.32	48.37	87.75	105.31
DUSP2	7.21	104.67	181.26	224.13
LRRC8D	2.34	5.08	11.18	14.06
ORM1	2.13	199.01	342.09	407.96
ORM2	1.52	70.27	127.67	151.74
PSTPIP2	4.62	17.70	45.84	47.52
TNFRSF4	0.22	2.29	4.75	5.74
WNT5B	0.20	1.97	5.92	6.27

Table 4.6. List of 22 TNFα-stimulated genes that were also upregulated, but to less extent by R848 alone. The combined treatment with R848 and TNFα was comparable to the effect of R848 alone indicating that R848 prevented their further induction by TNFα. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
CCDC134	1.23	3.23	6.92	1.96
CTSH	3.37	20.94	53.33	22.22
FAM110A	12.03	34.15	64.71	27.46
GHRL	8.98	24.95	48.64	18.71
GHRLOS	1.77	5.26	8.43	3.66
GPR35	0.20	2.78	6.14	1.77
GYPC	3.42	7.43	14.02	6.24
LOC389634	2.20	10.01	41.05	17.86
LRP5L	1.03	3.50	9.03	4.34
PHB	1.25	5.33	10.39	3.78
PLEC	3.32	12.45	24.71	12.32
PRDX1	3.80	21.72	33.21	13.99
PRPSAP1	2.56	22.77	40.91	19.18
SLC1A5	1.88	6.49	15.42	5.37
SLC25A39	1.40	4.64	6.81	3.40
SMPD2	4.38	10.67	23.80	9.22
TMEM205	5.52	35.53	87.78	35.69
TRAF2	1.22	2.85	5.95	2.52
AKR1A1	7.03	15.68	36.81	9.23
ANKRD22	0.77	3.66	8.57	1.88
CCL3L3	0.65	141.49	186.16	71.80
SPATC1	0.11	19.16	28.14	10.62

Table 4.7. List of TNFα-stimulated genes that were also upregulated, but to less extent, by R848 alone. The combined treatment with R848 and TNFα was lower than that induced by TNFα, but higher than R848-induced expression. Hence, R848 partially prevented their further induction by TNFα. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
C21orf67	0.57	1.93	10.22	4.12
FAM125B	0.10	0.52	3.62	1.52
FSCN1	2.55	12.48	113.69	45.90
LMNB2	0.49	1.03	7.60	3.56

Table 4.8. 19 R848/TNFα-stimulated genes that were downregulated when neutrophils were additionally treated with TNFα compared to untreated cells. TNFα stopped their maximum induction by R848. The fold change cut off for expression data was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ASB2	0.19	6.66	1.41	1.30
C19orf59	25.02	196.28	82.95	54.75
C1orf106	0.19	2.83	2.34	1.26
CDKN1A	7.97	906.56	290.85	372.75
EXOSC4	1.29	24.45	12.55	12.18
FAM83G	0.15	1.05	0.46	0.39
GPR4	0.39	2.35	0.99	0.78
HIVEP3	0.20	1.35	0.91	0.46
KCNH4	2.51	35.09	30.75	16.51
KIFC3	2.61	11.78	9.35	4.97
LACC1	0.53	29.71	16.35	14.65
NEDD4	0.61	3.27	2.48	1.40
PNP	1.75	10.42	4.30	4.96
PRR5L	19.79	121.75	54.70	53.33
TACSTD2	2.01	9.10	7.20	4.02
TNFRSF10D	1.06	8.38	4.58	3.49
TRIB3	0.61	5.57	2.02	2.68
CHMP7	5.58	12.33	10.15	5.64
SLC22A18AS	1.40	7.98	2.77	3.90

Table 4.9. 14 R848-stimulated genes that were downregulated to basal level in in untreated cells when neutrophils were additionally treated with TNFα. TNFα alone had no effect on these genes, hence TNFα prevented R848-mediated induction of gene expression. The fold change cut off for expression data was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
FAM27C	0.44	1.02	0.33	0.38
FAM54A	0.59	1.61	0.83	0.57
GSTT1	0.92	2.08	1.46	0.97
HPSE	26.95	95.75	24.18	33.39
INPP1	2.02	4.29	1.53	1.75
LIF	1.28	6.17	1.38	2.63
P2RY2	4.19	14.49	6.46	5.84
P2RY6	0.73	5.59	0.98	1.52
RALGAPA1	9.20	27.31	13.94	11.64
RPL21	0.05	19.76	0.06	0.07
SPRED2	2.27	5.79	3.43	2.55
TGM2	12.50	113.24	12.04	13.29
TMEM126A	0.89	2.18	0.91	0.82
TRMT6	0.43	1.88	0.76	0.70

Table 4.10. List of R848-stimulated genes that were downregulated when neutrophils were additionally treated with TNFα. TNFα partially stopped their maximum induction by R848. The fold change cut off for expression data was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
C1orf189	1.24	7.19	1.48	3.16
CD69	1.78	327.41	47.00	159.56
CKAP2L	0.17	7.67	1.30	3.50
CXCL1	96.22	587.14	91.83	226.30
FAP	0.18	10.39	0.88	5.07

Table 4.11. List of TNFα/R848-stimulated genes that were downregulated to basal level in untreated cells when neutrophils were treated with R848 and TNFα indicating that R848 and TNFα had a different combined effect to their solo actions. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
CYP1B1	1.06	5.05	5.02	2.36
LRG1	39.01	107.88	128.55	47.46
SEMA6B	18.13	47.58	63.14	19.95

Table 4.12. List of TNFα-stimulated genes (43 in total) that were downregulated when neutrophils were treated with R848 prior to TNFα. R848 alone did not regulate the expression of these genes, but it completely inhibited their TNFα-induced expression. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
B9D2	6.97	9.26	14.82	6.91
CGRRF1	1.90	2.51	4.12	1.95
COMMD3	8.00	8.80	17.53	6.51
FHOD1	35.67	39.55	76.17	37.66
GALNS	7.73	9.78	17.26	8.33
GPR137	2.48	2.70	6.09	2.50
GSTP1	2.02	2.23	4.85	1.68
PDE6D	5.09	6.40	10.25	4.31
PLIN5	5.86	9.65	11.75	5.54
RPIA	1.10	1.12	2.40	1.14
VPS37C	16.46	17.41	33.82	15.54
ZNF385A	3.53	4.74	14.21	3.94
CECR6	1.42	1.01	3.23	1.51
ETFB	5.44	5.35	14.06	6.21
NRSN1	1.08	0.92	2.58	1.26
PNKD	14.87	11.63	38.60	12.68
TNFAIP8L1	8.35	7.96	21.01	6.73
TNFRSF10B	59.33	54.15	122.80	61.13
ZNF281	8.68	6.79	24.17	7.30

Table 4.13. List of 9 TNFα-stimulated genes that were downregulated when neutrophils were treated with R848 prior to TNFα. R848 alone did not regulate the expression of these genes, but it partially inhibited their TNFα-induced expression. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
C6orf97	0.52	1.00	3.95	1.88
CDC14B	0.11	0.16	2.87	0.41
CDKN2C	0.35	0.39	3.44	1.44
GPX1	15.84	19.37	73.51	32.79
MAN1A1	7.61	14.14	48.32	17.48
NR6A1	1.93	2.59	11.14	4.44
TBC1D2B	11.62	23.10	63.65	29.92
TESK1	1.46	1.85	6.72	3.29
INO80B	0.36	0.27	5.38	2.04

Table 4.14. List of TNFα-stimulated genes that were downregulated by the treatment with R848 and TNFα. This downregulatory effect was due to R848 since R848 alone reduced the expression of these genes. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
GSN	75.93	34.15	157.58	46.98
LRRC61	1.61	0.55	4.34	1.37
PGLYRP1	8.02	3.88	18.39	3.21
RMRP	1.17	0.32	16.44	1.07
TICAM2	2.59	1.11	5.63	2.67

Table 4.15. List of selected TNFα-downregulated genes that were upregulated when neutrophils were treated with R848 and TNFα. The combined effect was comparable to that observed with R848 treatment indicating that R848 masked or prevented the downregulatory effect of TNFα. The genes were grouped based on R848 solo effect on their expression compared to no treatment. Number of genes is (38 and 42 and 12 respectively). 2-fold change cut off was applied to determine differential expression.

Gene	R848 effect	UT_{FPKM}	$R848_{FPKM}$	$TNFa_{FPKM}$	R848 + $TNFa_{FPKM}$
IFI44	Upregulation (IFN-response genes)	4.44	147.60	1.38	128.70
IFI44L		1.65	48.86	0.68	41.16
IFIT1		23.46	1249.30	7.63	1034.36
IFIT2		211.35	2279.85	42.66	2028.77
IFIT5		14.12	265.22	6.75	233.89
LY6E		3.82	35.49	1.42	29.85
MT2A		12.77	241.92	3.33	289.28
OAS1		5.04	109.77	1.93	95.40
OASL		21.35	222.58	4.00	196.72
PARP12		10.09	101.55	4.23	102.99
SP110		63.29	248.17	30.32	216.06
SP140		23.68	60.10	9.31	61.72
SP140L		8.97	34.97	4.40	32.26
TNFSF10		38.60	152.36	4.64	132.24
TRIM22		44.82	499.28	13.86	506.20
TRIM25		9.72	53.71	3.64	53.00
ZBP1		36.72	141.35	11.23	135.06
DYNLT1	No regulation	66.68	80.61	31.63	66.34
MIA3		12.57	15.95	5.38	13.11
NT5C2		73.19	71.81	30.17	75.68
PTP4A1		35.96	41.01	13.03	41.96
RNF138		12.31	10.78	4.41	9.77
TNK2		15.27	17.87	5.55	20.39
DYNLT1		66.68	80.61	31.63	66.34
HAUS4		30.42	8.98	3.39	7.05
PFKFB4	Downregulation	7.17	1.73	0.54	2.61
SLC30A1		7.97	1.83	0.79	1.74
TBC1D1		10.28	5.02	2.94	6.44
THBD		25.23	3.82	1.38	3.07

Table 4.16. List of 22 TNFα-downregulated genes (that were additively inhibited by the combined effect of R848 and TNFα. α. 2-fold change cut off was applied to determine differential expression.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
C18orf1	2.60	1.91	1.11	0.53
CAMKK1	24.71	3.72	6.86	1.42
CEACAM21	18.76	2.74	4.74	1.26
DEF6	58.67	13.72	15.58	6.30
EBPL	2.67	1.04	0.91	0.29
ENC1	34.87	17.22	12.97	3.16
EPOR	6.66	0.91	1.07	0.43
FAM211A	23.42	1.63	4.49	0.21
HIST1H4E	4.51	0.91	1.68	0.35
LYL1	33.89	4.39	5.66	1.31
MBD4	29.76	8.69	9.29	4.11
MSRB2	3.85	1.58	1.63	0.71
NLRP12	30.46	15.27	14.83	6.84
OGFOD2	2.39	0.64	0.96	0.23
OPRL1	7.56	0.76	2.38	0.36
POLR3K	10.17	0.63	0.87	0.18
PPP1R3D	17.49	4.57	6.03	2.03
SLC25A20	7.13	0.96	1.06	0.46
SNAI1	3.43	1.41	0.84	0.23
STAU2	3.32	1.08	0.82	0.36
TM6SF1	78.77	18.51	23.08	6.24
TMEM101	3.68	1.09	1.16	0.52

Table 4.17. List of some TNFα-downregulated genes (393 in total) that were further inhibited by treatment with R848 and TNFα. The joined influence of R848 and TNFα was comparable to R848-downregulatory effect which indicates that R848 masked the effect of TNFα. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ABHD5	52.20	7.22	16.18	7.88
AKT1	73.01	10.98	23.62	9.21
ARHGDIB	666.05	103.16	270.73	109.22
CTDSP2	112.17	23.70	54.11	26.91
CXCR1	362.03	14.29	38.75	13.18
CXCR2	665.42	31.11	92.81	31.40
EPB41	69.07	6.76	17.26	7.09
FCHO2	25.05	3.79	10.34	3.84
FURIN	73.06	17.49	34.56	15.19
H2AFV	32.97	9.10	15.73	7.48
HOTAIRM1	59.81	5.41	11.46	4.80
LIMD2	103.59	10.10	38.44	9.02
LMBRD1	80.09	12.41	27.35	13.48
LOC285074	26.56	4.85	12.75	5.69
MAP2K4	37.09	7.07	18.50	7.64
MAPK3	36.20	5.82	12.34	4.93
MYADM	98.94	16.77	46.60	14.55
NUAK2	58.24	3.96	18.11	4.54
ORAI3	24.74	5.85	11.00	4.90
OTUD1	36.63	6.10	13.74	6.49
PHOSPHO1	113.45	1.86	13.95	1.56
PYCARD	98.70	14.88	36.13	12.67
RAB11FIP4	39.09	6.30	11.66	5.21
RAB3D	64.01	4.70	16.56	4.22
RNPEP	38.42	8.37	15.88	7.51
SELPLG	111.40	3.12	11.61	2.61
SLC9A3R1	50.84	3.90	11.16	4.15
STK38L	40.26	8.44	17.54	8.59
TBC1D10C	51.50	2.83	10.56	2.53
TNFRSF10C	238.85	26.34	81.30	31.08
TUBA1A	48.38	5.68	12.50	4.83

Table 4.18. A list of 25 R848-downregulated genes that were also downregulated, but to less extent, by TNFα alone. TNFα appeared to partially stop the downregulatory effect of R848 since the combined effect of R848 and TNFα was comparable to or less than that induced by TNFα alone. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	$R848 + TNF\alpha_{FPKM}$
AMICA1	175.66	21.92	84.95	65.14
CDC42EP4	2.86	0.60	1.42	1.56
CERKL	4.47	0.37	0.58	0.85
CISH	2.35	0.76	1.13	1.94
CLEC9A	2.06	0.16	0.21	0.40
EGLN1	22.87	0.75	2.97	1.59
FAM45B	7.17	0.18	0.46	0.52
GLRX5	4.17	0.56	1.04	1.15
GPR27	2.69	0.17	0.47	0.44
GRAMD1C	11.36	0.69	3.31	1.78
HIST1H4D	4.23	0.17	0.71	1.15
HIST4H4	3.76	0.12	1.43	0.99
HLA-DMB	3.74	0.17	0.93	0.63
JARID2	54.48	5.72	13.98	12.60
KDM3A	38.44	8.17	18.03	22.85
MME	30.77	5.59	7.23	11.66
MYO18A	8.11	0.73	1.08	1.62
NFXL1	3.04	0.32	1.06	1.10
NMRAL1	3.66	0.11	0.25	0.34
PMF1	6.33	1.00	2.20	2.10
RAVER1	16.91	3.10	7.12	6.90
RGS2	742.35	72.00	176.34	188.35
SELO	11.57	0.88	2.86	1.96
TUBGCP3	9.14	0.82	3.78	1.90
ZNF362	3.93	0.07	0.24	0.28

Table 4.19. List of R848-downregulated genes that were further repressed by the combined effect of R848 and TNFα. R848 and TNFα together exhibited an inhibitory effect comparable to that induced by TNFα alone indicating that R848 effect was masked by TNFα. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ABAT	7.95	2.06	1.28	1.02
CDKN1C	2.73	1.01	0.59	0.48
MAP7	3.98	1.62	0.95	0.73
PCNA-AS1	9.53	3.58	1.80	1.27
PMM1	2.99	0.39	0.22	0.18
PRKDC	23.83	6.83	4.35	3.22
TXN2	9.07	3.98	2.94	1.96
VKORC1L1	3.08	1.17	0.56	0.57

Table 4.20. 12 R848-downregulated genes that were not affected by TNFα alone. TNFα appeared to partially stop the downregulatory effect of R848. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
BCORL1	2.56	0.33	2.08	0.99
CABIN1	3.72	0.41	2.31	0.82
DPM3	4.00	0.51	2.50	1.17
ELMO2	8.24	2.44	11.90	5.04
NAB2	3.13	0.32	1.93	0.72
SORD	4.39	0.91	6.22	2.09
CBX1	5.80	0.62	3.04	1.59
IGFLR1	17.90	3.03	12.28	6.19
KLF7	15.26	4.27	24.11	13.86
LOC100505746	18.26	5.93	25.88	13.56
PPP1R9B	67.24	24.09	114.10	62.37
WHSC1L1	14.94	2.45	9.83	4.94

Table 4.21. A list of 16 R848-downregulated genes that were not affected by TNFα alone. TNFα appeared to completely stop the downregulatory effect of R848. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
BAZ2A	43.49	14.76	28.08	31.24
CBFA2T3	3.15	0.76	3.66	5.63
CDC42SE2	92.21	42.52	87.78	88.18
FUT11	2.62	1.09	2.09	2.49
GTF2IP1	34.67	5.87	21.39	17.12
ITPKB	19.25	3.40	11.24	7.78
MAP3K5	12.50	5.91	9.61	19.01
NDUFA7	2.95	1.47	2.61	3.11
NFATC1	2.72	1.12	3.15	2.54
P2RY10	14.82	2.55	10.17	6.64
PHF12	38.92	10.13	39.72	31.99
STARD5	19.70	4.41	13.59	9.45
STAT5B	63.71	29.24	63.42	92.94
TP53INP1	110.33	18.60	67.81	54.22
TXNDC16	3.70	1.50	1.88	3.01
ZMYND8	4.35	0.92	3.13	2.36

Table 4.22. List of selected genes (212 in total) that were not differentially regulated by R848 or TNFα. However, the combined treatment with R848 and TNFα reduced their expression. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$TNF\alpha_{FPKM}$	R848 + $TNF\alpha_{FPKM}$
ARPC4	151.13	156.60	149.17	0.00
BPGM	13.80	13.55	12.32	5.85
C11orf31	8.37	6.61	8.00	3.91
CBFB	21.434	15.7673	15.8374	10.3837
CMTM3	15.80	15.05	17.83	7.61
DNAJA2	24.0494	22.6736	17.5616	11.6195
DNAJB14	18.6717	13.7166	14.4425	9.31058
DNAJC4	6.21	4.74	4.79	2.91
ELOF1	11.3922	8.06313	8.38527	5.6492
NUTF2	6.24	5.04	7.16	2.95
RGS19	107.174	75.086	86.665	36.2856
SF3A3	7.17344	5.74644	5.22519	2.22759
ST3GAL6	36.9171	26.8808	32.0108	15.4937
TLR1	99.2803	72.2701	84.2254	44.536
TTLL3	5.20	4.80	4.66	0.00
ZMAT5	15.7646	14.3698	11.2915	7.55661

Table 4.23. Patterns of gene expression regulation stimulated by R848 and/or GM-CSF. Up and down indicates upregulation and downregulation respectively, while No designates no differential regulation of gene expression. UT=no treatment, R= R848, G= GM-CSF and RG=R848+GM-CSF. 2-fold change cut off was applied to determine differential expression compared to unstimulated neutrophils. The following tables mention the members of each group of genes related to a particular pattern of transcription regulation indicated in this table. They are presented in the same order of their appearance in this table.

No. of genes	R/UT	G/UT	RG/UT	Comments	Possible pattern of regulation in RG
91	Up/No	Up/No	Up	RG>R+G	Synergistic upregulation
243	Up	Up	Up	RG=R+G	Additive upregulation
19	Up	Up	Up	RG=R, RG<G	R prevented further induction by G
18	Up	Up	Up	RG>G, RG<R	G partially prevented further induction by R
11	Up	Up	Up	RG=G, RG<R	G prevented further induction by R
248	Up	No	Up	RG<R	G partially stopped R-mediated upregulation
5	Up	Up	No		R and G had different combined effect to their individual effects
129	No	Up	No		R completely inhibited upregulatory effect of G
47	No	Up	Up	RG<G	R partially inhibited upregulatory effect of G
7	Down	Up	Up	RG<G	R partially inhibited upregulatory effect of G
12	Down	Up	Up	RG=G	G stopped the downregulatory effect of R
68	Down	Up	No		R completely inhibited upregulatory effect of G
69	Down	Up	Down	RG<G RG≥R	Downregulatory effect due to R alone to inhibit G effect
15	Up	Down	Up	RG<R	G partially inhibited upregulatory effect of R
9	Up	Down	Up	RG=R	R masked or prevented G effect and increased gene expression
32	Up	Down	No		G completely inhibited upregulatory effect of R
28	No	Down	No	RG=R	R prevented G effect
136	Down	Down	Down	RG<R, RG<G	Additive inhibition
9	No	Down	Down	RG<G	R further induced G effect
165	Down	Down	Down	RG=R, RG<G	R masked or dominated G effect
18	Up	Down	Down	RG=G, RG<G	G inhibited upregulatory effect of R and reduced basal level in UT
56	Down	No	No		G completely stopped downregulatory effect of R
46	Down	Down	Down	RG=G, RG<R	G masked or dominated R effect
30	Down	No	Down	RG>R, RG<G	G partially stopped downregulatory effect of R

No. of genes	R/UT	G/UT	RG/UT	Comments	Possible pattern of regulation in RG
91	Down	No	Down	RG<R	G enhanced downregulatory effect of R
2	Down	Down	Down	RG=G, RG>R	G inhibited further downregulation by R
397	No	No	Down		Only dual action of R and G reduced expression level in UT

Table 4.24. Genes synergistically upregulated by R848+GM-CSF. Molecule types, FPKMs associated with no treatment (UT), R848, GM-CSF and R848+GM-CSF treatments, and calculated synergy ratios were shown.

Gene	Molecule type	UT_{FPKM}	$R848_{FPKM}$	GM-CSF_{FPKM}	R848 + GM-CSF_{FPKM}	Ratio
CCL3		6.32	4704.98	53.59	9150.95	1.92
CCL3L1		14.40	1119.18	50.85	1448.68	1.22
CCL3L3		0.65	141.49	16.69	1195.45	7.53
CCL4		24.92	7463.63	121.76	10745.30	1.41
CCL4L2		55.20	7583.38	288.87	12567.30	1.59
CXCL2		5.04	112.72	13.30	172.59	1.32
CXCL3		1.08	9.97	2.57	16.47	1.21
IL1A	Cytokine	0.81	598.91	20.84	1516.00	2.44
IL1B		39.54	7529.71	353.23	13302.00	1.68
IL1RN		12.98	2426.58	2199.81	6532.34	1.41
IL36G		0.03	6.00	0.02	31.63	5.23
IL6		0.16	112.85	0.33	140.40	1.24
IL8		1602.37	30540.10	6931.77	49146.40	1.26
TNF		3.63	431.70	57.51	901.46	1.83
TNFSF15		0.75	9.61	3.35	31.97	2.33
AEN		0.28	1.52	1.88	13.03	3.54
ATP2C2		3.84	12.23	5.66	32.00	1.47
CARKD		0.81	1.66	2.40	6.12	1.26
CASP10		2.61	6.13	8.66	20.99	1.21
CASP5		1.12	18.82	5.12	48.39	1.93
CES1		0.52	5.59	2.26	19.73	2.36
DDX21		2.58	15.79	30.23	119.11	2.45
DDX47		1.69	1.01	1.45	6.48	1.56
DOT1L		0.91	17.32	5.68	28.89	1.21
ECE2		0.02	0.06	0.93	13.86	13.61
NDUFV2	Enzyme	12.38	80.28	104.00	496.34	2.52
PRDM7		0.25	2.34	0.31	7.37	2.55
RDH13		0.89	3.70	1.38	10.28	1.72
RHOH		1.89	14.53	100.72	279.75	2.39
RNF175		0.33	6.64	3.17	18.63	1.84
SOCS3		15.55	65.11	257.06	754.64	2.23
TARP		0.93	1.47	11.11	39.72	2.94
TRIB3		0.61	5.57	3.21	17.96	1.91
TXN		50.17	194.65	457.84	846.49	1.20
UPP1		4.40	24.19	186.02	272.17	1.27
USP36		0.97	2.27	3.86	9.34	1.32
GNL2	GTPase	1.99	3.45	4.98	17.21	1.65

Gene	Molecule type	UT_{FPKM}	$R848_{FPKM}$	GM-CSF_{FPKM}	R848 + GM-CSF_{FPKM}	Ratio
GNL3		0.49	0.97	2.33	5.09	1.34
GTPBP4		0.74	2.81	12.53	23.17	1.44
NOLC1		1.12	3.45	3.75	15.02	1.81
RHOU		0.12	16.25	0.86	45.07	2.62
CFLAR	Enzyme regulator	75.94	286.59	192.55	714.49	1.29
PPP1R15A		50.41	851.04	109.62	1325.18	1.31
CD40	Receptor	0.01	2.85	0.22	7.56	2.44
CD44		62.09	1050.03	945.92	2524.10	1.23
CD48		15.66	352.33	38.18	577.42	1.42
SLAMF1		0.14	2.58	0.54	65.06	19.95
HRH1		0.29	6.55	0.63	11.23	1.50
P2RX4		1.44	11.74	4.34	48.57	2.77
SDC4		0.02	0.85	0.26	7.09	6.31
TFRC		1.19	32.83	3.55	46.17	1.23
TLR2		14.49	237.30	215.56	941.23	2.01
PTX3		0.09	4.42	1.25	11.46	1.99
CCND2	Cell cycle regulator	2.56	0.66	18.65	52.71	2.41
CCNE2		1.00	1.04	6.87	31.97	3.59
CDK4		1.22	2.55	3.66	11.83	1.59
DNTTIP2	Transcription regulator	12.21	79.82	48.83	186.67	1.33
C6orf108		0.35	4.34	0.33	8.32	1.66
EGR3		0.69	39.48	15.14	72.12	1.30
ELK1		1.78	7.53	3.40	18.97	1.49
ETS2		20.75	200.95	303.75	674.40	1.28
EYA3		6.51	33.12	31.97	87.80	1.23
FOSL1		0.17	5.09	1.95	15.69	2.18
IFT57		0.77	7.37	0.63	11.77	1.34
MAFF		7.94	270.87	79.53	487.12	1.36
NR1D1		1.19	8.49	4.13	18.46	1.34
ABCF1	Translation initiation factor	5.20	20.55	16.32	56.82	1.35
EIF1B		53.68	236.16	304.92	745.91	1.25
EIF3I		4.98	10.28	13.37	36.59	1.28
EIF4A1		21.90	64.63	89.72	251.60	1.43
TRMT6		0.43	1.88	3.62	12.97	2.19
B7H6	Structural molecule	0.50	34.77	1.95	62.66	1.68
MRPL14		0.30	4.70	1.15	11.38	1.85
MRPL52		1.97	19.69	2.44	36.63	1.52
MRPS24		1.10	10.43	2.63	19.03	1.34
PI3	Inhibitor	28.47	7373.06	240.17	16353.10	2.14
SERPINB2		0.24	11.71	0.91	24.82	1.93

Gene	Molecule type	UT_{FPKM}	$R848_{FPKM}$	GM-CSF_{FPKM}	R848 + GM-CSF_{FPKM}	Ratio
SPINK1		1.89	16.40	17.14	46.95	1.32
SPINT2		5.52	61.78	124.21	278.85	1.46
SLC25A33	Transporter	0.71	2.70	2.98	11.35	1.77
SLCO4A1		0.61	11.60	0.88	19.94	1.52
EDN1	Hormone	0.57	5.15	2.31	48.73	6.06
F3	Coagulation factor	0.85	30.72	1.60	48.55	1.46
GJB2	Junction Channel	0.27	8.51	0.14	13.80	1.55
HBEGF	Growth factor	0.36	0.65	17.78	63.96	3.40
HSPA9	Heat shock protein	4.53	9.26	16.70	43.59	1.43
UBQLN4	Proteasomal ubiquilin	0.67	1.95	1.06	12.25	3.33
RPF2	rRNA binding	0.09	2.39	1.51	15.62	3.92
TRAF1	Signal transducer	0.73	273.02	2.28	421.43	1.53
COMMD4	Other	2.43	9.22	5.54	27.72	1.61

Table 4.25. A selection of GM-CSF-upregulated genes that were additively induced by R848 effect (243 genes). The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	GM-CSF_{FPKM}	R848+GM-CSF_{FPKM}
AZIN1	34.64	389.25	97.77	493.22
CREM	21.12	65.04	49.95	128.68
EIF1	156.90	544.42	573.28	1260.22
FPR2	201.72	736.18	810.62	1675.96
GLA	19.97	80.66	41.85	142.25
GNG2	30.69	677.51	80.10	762.68
MAPK6	2.88	73.06	8.30	82.27
NFKBIZ	14.12	774.06	61.80	823.24
OLIG1	0.61	13.53	6.78	20.58
PIM3	32.78	359.94	212.34	571.99
PLAU	43.59	1397.04	233.17	1649.84
RBM17	3.52	24.61	11.36	38.25
SAMSN1	17.41	610.02	68.98	693.21
SERPINB8	7.71	107.14	17.76	127.87
TREML3	6.55	23.65	27.38	55.73
TRPM2	2.58	24.30	6.65	32.44

Table 4.26. List of 19 GM-CSF-upregulated genes that were downregulated by the addition of R848 although R848 alone induced their expression. The expression level of the genes in response to R848 and GM-CSF was comparable to or slightly lower than that induced by R848 alone indicating that R848 possibly inhibited further induction of expression by GM-CSF. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ABP1	1.08	7.13	14.46	4.40
ANKRD22	0.77	3.66	80.92	1.96
CBWD3	1.34	2.69	5.52	2.43
CBWD6	0.62	1.33	2.82	1.20
CD97	63.72	131.68	624.79	236.55
DUSP6	20.25	109.80	383.40	147.63
FAM65B	21.68	63.51	236.88	101.67
HLA-DQB1	9.98	36.48	89.79	39.53
HLA-DQB2	0.32	1.07	4.12	1.11
IL18R1	2.80	7.29	29.73	6.18
IL1R2	44.35	163.41	635.04	191.71
LRG1	39.01	107.88	300.75	119.11
POLA2	1.09	3.77	7.70	3.81
SEMA6B	18.13	47.58	115.43	45.68
TARS	1.66	4.27	8.80	4.12
TMX2-CTNND1	0.41	0.97	2.83	1.12
C12orf59	0.25	1.44	4.13	0.60
CEACAM1	17.36	84.28	181.43	35.52
MAP2K6	0.27	1.28	4.32	0.59

Table 4.27. List of 18 genes that were upregulated by GM-CSF or R848. However, the combined effect of the two stimuli was less than that observed with R848 effect alone indicating that GM-CSF partially inhibited R848-induced effect. The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ASB2	0.19	6.66	0.77	3.21
ATF3	0.40	8.52	0.81	3.03
BTG3	0.26	22.79	0.69	10.98
C20orf118	0.74	13.99	1.71	3.70
CCL18	0.03	21.69	0.42	5.43
CCL2	0.30	64.84	4.10	8.82
DDX60L	16.17	418.19	87.54	203.80
GPR35	0.20	2.78	0.46	1.08
KCNH4	2.51	35.09	5.33	12.71
LAMP3	0.56	51.75	1.65	9.55
LIMS1	9.09	334.43	33.14	72.86
LIMS3	3.12	92.16	13.09	35.63
NR4A1	0.31	5.67	0.96	2.23
PLSCR1	7.17	505.74	95.25	242.59
SAMHD1	8.05	267.08	28.23	77.30
SMAD3	0.75	29.79	2.00	14.50
SPATC1	0.11	19.16	1.35	7.58
TFEC	0.66	167.54	2.63	53.75

Table 4.28. List of 11 R848-upregulated genes that were downregulated by the combined treatment of R848 and GM-CSF. Genes' level was increased by GM-CSF alone, but GM-CSF inhibited further induction of the genes by R848. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ARHGAP24	0.12	3.23	1.40	0.91
C5orf58	0.54	5.46	1.98	1.78
CYBB	20.65	353.70	161.16	168.07
DYNLT3	5.15	43.73	12.09	19.74
GNG10	44.65	136.84	93.72	65.08
LGALS8	2.68	7.72	5.84	3.35
LMNA	0.17	1.70	0.48	0.55
MILR1	2.44	13.54	5.96	5.44
RFX5	2.62	10.47	8.15	5.00
TLR4	84.78	298.97	175.11	144.13
TRIM22	44.82	499.28	97.53	113.51

Table 4.29. List of a selection of R848-upregulated genes (248) that were downregulated by the addition of GM-CSF. The genes were not differentially regulated by GM-CSF alone. The fold change cut off for expression data in all comparisons was 2-fold. Majority of genes in this category are IFN-response genes.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
BIRC2	37.03	191.92	36.81	86.80
CYBA	379.26	1527.56	430.50	694.34
DMXL2	7.76	248.47	7.36	113.86
EIF2AK2	6.17	220.04	5.35	62.64
FAM129A	75.45	328.99	79.74	159.49
GAPT	83.65	641.69	84.88	241.24
GNB4	19.01	40.44	17.58	17.47
GTPBP2	11.33	79.50	11.43	33.02
HIF1A-AS2	126.25	610.39	143.31	175.60
IFI6	50.76	1295.02	50.87	262.31
LGALS9	33.42	82.45	30.51	23.70
OAS3	6.12	499.06	6.11	187.54
ORM2	1.52	70.27	1.26	12.46
PARP12	10.09	101.55	8.93	39.42
PARP9	25.80	307.15	20.87	95.26
PLAC8	4.18	43.13	3.89	11.43
SAMD9	44.58	290.02	36.44	70.44
SP110	63.29	248.17	72.22	100.64
SRC	1.03	77.48	1.17	19.87
STAT2	16.82	201.23	20.09	71.77
TRAFD1	27.94	61.62	27.82	18.91
TRIM5	10.03	75.41	10.47	18.95
UBL3	29.14	69.04	30.52	23.41

Table 4.30. List of 5 GM-CSF-upregulated genes that were downregulated by the addition of R848 although R848 alone induced their expression. R848 or GM-CSF-induced expression was reverted back to the basal level in untreated (UT) cells by their combined effect. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
MB21D1	2.09	7.03	10.76	2.82
RPL21	0.05	19.76	16.71	0.03
SSB	1.62	5.27	5.92	2.31
TGM2	12.50	113.24	60.29	21.74
TXNIP	336.38	807.82	873.32	288.71

Table 4.31. List of a selection of GM-CSF-upregulated (129) genes that were downregulated by the treatment with R848 and GM-CSF. The genes were not differentially regulated by R848 alone, but R848 reduced their expression to the basal level in unstimulated (UT) cells The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ABCG1	13.61	14.11	36.36	15.35
BCL9	0.97	0.90	10.28	1.19
C1RL	9.08	7.32	34.13	9.33
C4orf3	12.24	13.13	53.89	14.70
DDAH2	22.41	27.92	49.97	20.51
EIF4G3	7.71	6.49	15.62	6.69
F2R	2.86	2.39	11.68	2.56
FKBP1A	129.21	131.35	276.55	135.67
IL18RAP	7.57	8.73	39.62	8.12
IL1RAP	63.81	53.78	254.57	51.03
IRF2BP2	49.44	50.54	193.03	49.30
KLF2	41.12	45.88	107.88	45.41
NOTCH2	36.43	50.15	86.69	42.02
RGS3	5.44	6.61	18.20	4.92
RNF141	34.63	28.42	120.09	28.49
SWAP70	7.64	6.47	16.50	6.25
TSPAN14	32.61	34.55	67.96	29.53
TUBA1C	16.67	14.10	45.39	12.93
TUBB	29.12	23.87	82.65	39.39

Table 4.32. List of some GM-CSF-upregulated genes (47) that were downregulated by the treatment with R848 and GM-CSF. The genes were not differentially regulated by R848 alone, but R848 partially reduced their expression compared to GM-CSF-induced effect. to less than the basal level in unstimulated (UT) cells The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ANXA3	3.05	5.79	50.10	24.38
C6orf97	0.52	1.00	13.06	3.08
CBWD1	1.06	2.03	6.40	2.65
HRH4	0.84	1.62	181.42	18.97
ITGAM	54.12	78.35	315.06	139.99
NUBP1	0.87	1.25	22.62	9.26
PER2	0.91	1.69	13.14	2.68
POU5F1P3	3.89	5.48	52.80	18.54
RMND1	0.91	1.65	10.55	4.72
SERTAD2	8.63	13.02	103.98	46.53

Table 4.33. List of 7 GM-CSF-upregulated genes that were partially downregulated by the treatment with R848 and GM-CSF compared to untreated cells. The expression of the genes was decreased by the effect of R848 alone. The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ACAA2	1.07	0.44	9.94	4.52
CISH	2.35	0.76	573.28	207.68
FLT3LG	1.96	0.37	10.86	5.35
SNTB1	0.81	0.23	12.08	4.56
SORT1	2.76	0.50	40.46	10.16
STEAP4	30.47	6.41	551.15	116.37
TGFA	8.30	2.01	64.33	20.47

Table 4.34. List of 12 genes with reduced expression by R848. All genes were upregulated by GM-CSF alone. However, the combined treatment of R848 and GM-CSF was similar to that induced by GM-CSF alone indicating that GM-CSF stopped the downregulatory effect of R848. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ACSS2	11.80	1.51	52.24	30.69
CAMK1D	17.57	8.44	50.51	28.87
CD300C	3.52	1.68	10.51	5.38
GALNT2	4.06	1.15	12.26	8.73
HSPA1A	31.40	13.17	80.10	72.10
HSPA1B	21.08	9.18	59.78	53.34
LOC100506963	2.19	0.72	4.48	4.11
NUDT5	6.89	2.94	62.82	34.15
RRP12	2.53	0.81	10.57	8.72
SUMF1	2.18	0.72	4.96	3.44
TSTA3	6.67	3.11	14.62	9.24
USP32	33.97	15.30	81.20	48.63

Table 4.35. List of selected GM-CSF-upregulated genes (68 in total) that were downregulated by pretreatment with R848 to the basal expression level in untreated cells. The expression of the genes was decreased by the effect of R848 alone. The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
BST1	29.76	13.06	90.29	24.59
CCND3	25.56	8.26	104.23	20.12
CTSC	94.48	30.06	611.55	110.54
HIATL1	13.41	6.50	31.60	10.82
KIAA0040	21.94	5.53	219.57	18.98
KIF21B	9.19	3.14	28.94	7.72
LOC283070	10.51	2.99	26.27	12.25
MAPKAPK3	37.73	4.97	185.38	27.04
PRDX3	6.44	2.79	14.47	4.53
PTEN	51.51	20.30	137.31	37.55
PTENP1	11.53	4.55	35.06	10.24
SNX17	7.19	3.33	14.51	5.92
WDR37	8.26	2.57	18.09	7.53

Table 4.36. List of selected GM-CSF-upregulated genes (69 in total) that were downregulated by pretreatment with R848 to less than the basal expression level in untreated cells. Expression of genes was decreased by the effect of R848 alone. Fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
AFF1	18.85	9.40	43.54	7.81
CAB39	62.14	25.43	233.62	28.48
CMTM1	20.10	7.92	61.90	9.36
GLIPR2	86.05	23.73	213.92	30.28
LPIN2	88.00	15.74	219.95	13.34
LTA4H	43.57	6.48	231.93	11.82
MYADM	98.94	16.77	206.30	18.68
P2RY13	198.60	31.45	437.99	40.27
PGAM1	29.39	6.74	59.36	11.21
PIK3CD	83.56	28.69	181.00	35.79
PPM1M	33.35	7.22	71.60	8.42
PYCARD	98.70	14.88	219.41	29.74
STK17B	43.02	15.32	86.86	9.69
TSPO	65.14	9.01	150.40	13.16
TUBA1A	48.38	5.68	122.50	7.69
TUBA1B	28.69	9.08	84.34	10.38
TWF2	64.37	17.22	131.03	14.26

Table 4.37. Genes that were reduced by GM-CSF. Their expression was increased by R848 alone. GM-CSF either partially diminished the upregulatory effect of R848 (15 genes,top group) or had no effect on R848-induced expression (9 genes, bottom group). The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
CMPK2	2.51	220.22	1.18	38.26
DDX58	25.14	217.55	12.10	58.22
DUSP16	6.07	45.96	2.48	15.08
FSCN1	2.55	12.48	1.22	5.61
HERC5	7.78	349.25	2.42	37.27
IFI44	4.44	147.60	1.95	25.21
IFIT1	23.46	1249.30	8.62	186.02
MT2A	12.77	241.92	5.04	67.40
MX2	79.53	650.57	33.39	206.88
OAS1	5.04	109.77	1.73	22.58
PTGES	7.10	488.59	0.96	46.30
TCFL5	2.70	211.85	0.82	85.86
IFIT1B	0.56	22.42	0.17	2.87
KIAA1045	0.47	3.22	0.09	1.24
MARCH3	0.97	12.34	0.32	3.29
BCL9L	2.49	10.03	0.65	8.54
BMF	5.12	16.31	2.35	10.96
CLEC4E	64.15	509.90	30.35	334.33
SLC7A7	9.96	196.48	4.60	191.63
TECPR2	16.41	40.80	7.59	40.81
GRAMD1A	32.39	471.13	15.25	315.20
HMGN2P46	4.86	12.19	1.33	11.58
LPCAT1	160.02	554.34	65.44	368.33
PLAUR	251.99	1435.37	89.39	905.31

Table 4.38. List of 32 genes that were downregulated by GM-CSF. The expression of the genes was increased by R848 alone. GM-CSF completely inhibited the upregulatory effect of R848 and their levels were similar to that of the untreated cells. The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ANXA6	1.52	5.20	0.73	1.78
AOAH	20.10	50.22	3.95	18.41
APOL3	1.31	4.75	0.42	0.97
C1GALT1	9.43	45.63	3.22	7.56
CADM4	6.90	17.19	1.53	7.30
COL9A3	2.58	9.82	0.58	3.15
DNAJB5	0.99	4.80	0.22	1.39
GBP1	34.95	323.58	11.41	49.95
GBP1P1	3.19	32.25	1.09	5.40
GBP3	4.41	40.45	2.06	6.78
GBP4	31.57	130.32	5.68	15.65
GBP7	0.90	4.14	0.12	0.56
IFIT2	211.35	2279.85	74.69	269.75
LIF	1.28	6.17	0.35	1.21
LOC100132707	1.37	4.51	0.23	2.12
JAG1	2.96	10.27	1.44	5.55
MEPCE	17.79	40.12	7.76	18.28
OASL	21.35	222.58	4.51	36.87
OTUD5	43.76	97.28	20.76	72.94
PDE4A	0.68	2.65	0.33	0.97
PDE8A	2.51	5.96	0.93	2.90
PLXNC1	101.79	274.68	43.69	128.73
RNF144A	1.87	5.12	0.73	1.91
SELK	32.80	77.09	10.01	51.03
SERPING1	2.42	7.84	1.20	2.54
SGMS1	3.28	10.03	0.74	3.46
SIN3A	7.29	16.51	2.97	12.01
SSBP3	3.97	11.25	1.95	5.29
SLC25A39	1.40	4.64	0.56	1.45
STAT5A	23.80	63.10	8.11	38.48
TMEM123	54.57	364.77	19.27	64.54
TP53INP2	34.18	90.32	11.32	46.86

Table 4.39. List of selected GM-CSF-downregulated genes (28 in total) that were returned to the basal level of gene expression in untreated cells by the effect of R848. R848 alone had no effect on these genes. The fold change cut off for expression data in all comparisons was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
AATK	40.04	51.54	16.60	34.97
ACD	5.90	3.24	1.19	5.06
BASP1	324.44	535.68	87.68	202.12
C7orf53	16.89	12.34	6.69	16.70
CABLES2	2.61	4.42	0.51	2.51
CERS4	5.51	6.37	1.45	3.16
CHD4	16.68	28.62	8.12	21.02
CHRNA10	2.72	2.77	0.80	2.28
FAM134B	2.12	2.47	0.74	2.60
FOXP1	3.14	3.36	1.06	3.70
GPR137	2.48	2.70	0.88	2.26
GPR157	3.77	3.58	0.89	3.34
HSH2D	85.19	148.10	21.18	50.67
NCOA4	220.92	240.50	59.12	144.35
NR4A2	2.50	2.34	0.63	2.79
RAD9A	9.76	8.90	4.34	10.38
REPS2	7.62	10.51	2.57	6.43
RNU6ATAC	16.56	26.77	7.97	20.79
SIPA1L1	24.39	24.64	9.70	19.60
SLC37A1	4.39	3.09	1.23	2.91
STX5	16.74	21.63	7.48	19.15
TESK2	5.23	9.68	1.19	3.78
TFDP1	30.36	50.23	8.69	24.81
TMEM8A	28.83	33.23	11.05	23.56
UBR7	3.06	1.66	1.17	2.78
VPS13D	3.04	3.74	1.52	3.41
WDFY1	12.66	18.29	4.61	11.60
ZSCAN16	5.43	9.04	1.19	3.28

Table 4.40. A selection of GM-CSF-downregulated genes (136) that were additively inhibited by the combined effect of R848 and GM-CSF. R848 alone reduced the expression of these genes. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ABHD2	126.68	22.00	27.61	8.08
AMPD2	281.33	51.57	81.23	15.66
BTG1	265.07	76.63	57.22	27.37
BTN3A1	104.50	20.70	27.10	8.25
C14orf159	48.91	10.72	14.59	3.51
CCNG2	89.78	15.89	13.31	4.22
CDKN1B	122.62	11.27	44.36	2.95
CXCR2	665.42	31.11	168.04	13.47
FCGR3A	621.82	143.48	218.89	60.09
FCGR3B	1287.33	303.13	448.65	119.29
FGL2	109.93	15.76	47.26	4.81
HIST1H2AC	211.50	29.66	94.78	10.92
INPP5D	159.58	43.34	60.16	17.85
LPGAT1	44.86	12.95	18.92	5.97
MKL1	66.00	11.69	23.25	4.61
MNDA	587.22	160.98	262.18	80.16
NUP50	81.03	18.23	37.55	6.82
PHF12	38.92	10.13	17.68	2.58
RERE	56.35	13.66	21.23	1.25
RGS2	742.35	72.00	195.59	34.55
TLR8	52.82	14.37	16.14	6.61
TM6SF1	78.77	18.51	35.39	5.57
TMEM66	385.63	47.07	101.52	22.91

Table 4.41. GM-CSF-downregulated genes (9) that were additively inhibited by the combined effect of R848 and GM-CSF. R848 alone had no effect on the expression of these genes. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
C7orf70	12.50	8.38	4.53	1.66
DCLRE1C	5.34	4.65	2.43	1.12
GPR160	86.64	57.69	30.89	10.80
IPMK	23.86	18.24	10.77	3.53
PCMTD1	33.69	28.45	16.13	7.54
RNF138	12.31	10.78	5.53	2.11
SPOPL	52.38	32.76	22.19	8.00
TRAF3IP3	41.83	34.07	19.10	6.07
TRANK1	95.83	93.92	33.05	14.31

Table 4.42. List of some GM-CSF-downregulated genes (165) that were further inhibited by R848. The joined influence of R848 and GM-CSF was comparable to R848 effect alone. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
AKT1	73.01	10.98	35.46	9.79
CXCR1	362.03	14.29	85.81	9.60
DEF8	64.50	3.69	31.03	3.01
FKBP8	120.56	23.37	59.62	19.16
HMGN2	90.74	12.96	33.89	12.42
ITGAL	33.41	6.81	16.19	6.20
MPPE1	35.68	3.46	12.66	3.50
NOTCH1	88.32	21.62	42.66	18.19
PACS1	46.20	2.46	14.17	2.52
RNASE6	158.26	3.27	25.92	2.80
SLBP	31.80	1.25	14.73	1.25
SRPK2	42.47	1.57	10.39	1.38
TACC3	48.11	1.56	20.76	1.29
TBC1D10C	51.50	2.83	20.14	2.39

Table 4.43. List of 18 genes that were upregulated by R848. All genes were downregulated by GM-CSF. However, the combined treatment of R848 and GM-CSF was lower than the basal level in untreated (UT) neutrophils. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ARL15	2.53	5.36	0.57	0.51
BMPR2	0.95	2.17	0.29	0.37
CUX1	12.68	30.35	5.20	3.49
CYFIP2	50.56	127.26	14.66	15.45
EXOC3L1	0.69	2.48	0.11	0.21
FAM8A1	46.67	128.53	17.98	17.08
GBP5	104.51	218.21	50.95	98.23
GM2A	2.61	13.84	0.99	1.88
LOC100861402	0.81	2.19	0.39	0.46
MAK	2.80	6.44	1.38	2.25
PIGF	34.12	77.51	16.55	14.47
RALGAPA1	9.20	27.31	3.55	6.87
SH3D21	9.69	25.65	4.17	7.99
SIGLEC10	159.78	370.09	55.52	80.44
SIGLEC11	5.97	12.00	2.53	2.93
STX17	2.87	7.07	1.25	1.65

Table 4.44. A selection of 56 genes that were downregulated by R848. GM-CSF and the combined treatment had no effect on these genes indicating that GM-CSF completely stopped the downregulatory effect of R848. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ARRB2	381.09	178.63	493.90	397.27
BCYRN1	13.13	4.70	13.61	11.43
COX5A	6.38	1.77	6.36	4.75
INTS8	15.01	4.30	19.85	20.82
MFNG	82.42	19.24	73.50	67.66
PITPNC1	14.67	5.60	27.56	20.97
PPP1R35	14.05	5.19	14.35	10.81
PSD4	67.14	26.53	78.88	56.33
XRN2	18.29	7.86	25.04	23.72

Table 4.45. List of some R848 -downregulated genes (46 in total) that were further inhibited by combined treatment of R848 and GM-CSF. The joined influence of R848 and GM-CSF was comparable to GM-CSF downregulatory effect indicating that GM-CSF masked R848 effect. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ALG1L2	13.89	4.43	3.09	1.87
BAD	6.38	2.14	1.49	0.91
CCDC28A	34.49	6.68	4.09	2.73
CPNE3	7.38	3.35	2.16	1.30
DCAF15	13.42	5.60	4.07	2.69
FAM118A	16.48	3.85	1.33	0.91
FRY	17.19	4.11	1.93	1.75
MEGF9	83.13	39.30	10.87	9.43
NUMA1	22.91	8.59	4.41	3.18
PLOD1	11.41	4.22	1.25	0.84
SMC4	7.67	2.79	1.69	1.25
ST6GALNAC2	25.52	4.98	2.49	1.89
TMEM63A	5.41	2.69	1.71	1.29
UCK1	6.78	2.91	2.15	1.35

Table 4.46. A selection of 30 genes that were downregulated by R848. GM-CSF alone had no effect on these genes, but it partially inhibited the downregulatory effect of R848. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ATP2A3	93.11	8.79	103.78	22.82
BCKDHA	5.36	1.20	8.79	2.55
CMTM2	85.21	11.10	118.69	22.65
CNPPD1	25.60	4.83	33.27	10.60
DUSP23	6.71	0.96	7.02	2.50
ERICH1	5.26	1.20	6.86	2.54
FAM101B	29.10	4.38	37.74	12.53
MINK1	13.43	2.35	19.68	8.92
MLX	18.12	5.86	26.82	11.96
MMP25	207.35	12.09	180.08	27.40
NAPRT1	28.06	6.47	40.84	18.12
PGM2	7.68	1.16	11.89	4.10
PMF1	6.33	1.00	10.18	2.17
RALBP1	37.65	3.24	31.50	14.89
RNF220	5.87	1.36	8.62	3.80
SLC19A1	57.22	5.30	64.56	10.98
THBD	25.23	3.82	32.00	9.61
TMC6	34.38	1.77	29.22	7.40
TMEM65	10.10	1.17	10.88	2.51

Table 4.47. List of selected R848 -downregulated genes (91 in total) that were further inhibited by the combined treatment of R848 and GM-CSF. GM-CSF alone had no effect on their expression, but it augmented R848-induced effect. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
C10orf54	213.24	45.30	193.09	12.52
CEACAM21	18.76	2.74	15.47	1.02
CLEC2B	97.15	44.62	101.32	20.95
EVI2B	450.08	86.82	462.89	39.46
FLI1	72.87	14.15	75.93	6.75
PHF23	21.70	8.87	17.77	4.22
RSBN1L	27.77	6.56	22.78	1.65
SETD1B	27.79	5.93	22.71	2.21
TSEN34	27.95	3.10	27.12	1.50
ZNF217	67.65	24.99	65.59	11.85

Table 4.48. Genes downregulated by R848 or GM-CSF. However, the combined treatment of R848 and GM-CSF was similar to that induced by GM-CSF alone indicating that GM-CSF prevented R848-induced effect. The fold change cut off for expression data in all comparisons was 2-fold in both directions.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
HMGB1	48.86	11.24	24.15	22.92
TP53INP1	110.33	18.60	37.75	43.27

Table 4.49. List of selected genes (397 in total) that were not differentially regulated by R848 or GM-CSF. However, the combined treatment with R848 and GM-CSF reduced their expression. The fold change cut off for expression data was 2-fold.

Gene	UT_{FPKM}	$R848_{FPKM}$	$GM\text{-}CSF_{FPKM}$	$R848+GM\text{-}CSF_{FPKM}$
ATP7A	14.10	11.27	11.36	6.15
CMIP	46.98	36.54	35.30	20.83
CSGALNACT1	11.62	13.01	14.31	4.15
DCAF12	21.90	17.10	26.39	8.97
GLRX	102.56	86.69	84.39	33.58
IRF1	92.67	132.39	85.46	33.07
ISCU	70.47	70.21	56.82	34.73
LSP1	641.83	548.49	581.96	310.23
PIK3CA	17.93	15.46	16.60	8.53
PLEKHO1	116.49	93.86	106.90	47.92
PSMB9	92.79	104.39	98.99	40.43
RAB27A	27.60	23.25	37.79	12.88
RNASEL	24.25	22.89	26.72	10.83
RUNX3	10.02	12.63	10.76	4.39
SLA	176.51	200.82	133.17	78.18
SLC12A6	102.53	107.00	114.48	0.00
SNX13	14.97	12.71	12.13	6.59
TP53I11	19.35	15.71	19.36	6.54
USF1	65.35	59.12	53.10	25.70